
Nutritional evaluation of santol (*Sandoricum koetjape*) and the effects of santol flesh extract on *Drosophila melanogaster*

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Abstract Santol (*Sandoricum koetjape*) fruit was low in calories and deficient in vitamins B1, B2, and C, but it is a rich source of potassium, containing 156.05-188.48 mg per 100 g. The first filial (F1) generation was observed on the 3rd day after emergence, and survival rates exceeded 90% at all concentrations. In climbing assays, a 100 mg/mL concentration reduced climbing speed over a 10 cm distance by 9.35 times in the P generation and 7.74 times in the F1 generation compared to control. At this concentration, the adult emergence rate decreased to 53%, and body weight in both generations was reduced. However, no difference in body weight was observed at concentrations of 20, 40, 60, and 80 mg/mL compared to controls. These findings indicated that santol fruit is a good source of potassium, but high concentrations of santol extract negatively impact the health and performance of *D. melanogaster*.

Keywords: *Drosophila melanogaster*, Santol (*Sandoricum koetjape*), Santol extract, Nutritional value

Introduction

Santol (*Sandoricum koetjape* Merr.) is widely consumed in Southeast Asia for its distinctive flavour and associated benefits. The fruit is nutritionally rich, containing polysaccharides, natural antioxidants, and vitamins. Sour fruits like oranges, lemons, pineapples, and tamarinds are typically high in organic acids, such as ascorbic acid (vitamin C), citric acid, malic acid, and tartaric acid, which contribute to their tangy flavor and provide health benefits, including cell protection, enhanced iron absorption, and immune support (Shi *et al.*, 2022). Variants of santol, such as the yellow variety (Honduras) and unspecified types (India), have demonstrated notable nutritional profiles, as described by Morton (1987). According to this source, 100 g of santol provides 4.30 mg of calcium,

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17.40 mg of phosphorus, 0.42 mg of iron, and 86 mg of vitamin C, along with trace amounts of thiamine (0.045 mg) and niacin (0.741 mg).

Phytochemical studies have identified various bioactive compounds in different parts of the santol plant, including fruits, seeds, leaves, and bark. For example, methanolic extracts from the flesh and peel contain tannins, coumarins, and betacyanins, while saponins are detected only in the flesh, and alkaloids are present in the peel (Poeaim and Pedklang, 2024). These bioactive compounds demonstrate significant medicinal properties, including antimicrobial, anti-inflammatory, and anticancer effects. The stem bark extract has shown anti-inflammatory activity in animal models and induces apoptosis in colon cancer cells (Rasadah *et al.*, 2004; Nassar *et al.*, 2012). Additionally, triterpenes such as ketonic acid and sandorinic acid have been shown to exhibit cytotoxic effects against various cancer cell lines (Wijaya, 2022). Tannins in santol further enhance its anticancer potential by inhibiting cancer cell proliferation and inducing apoptosis (Kleszcz *et al.*, 2023). The leaf extract is antibacterial, particularly against *Staphylococcus mutans* (Pambudi *et al.*, 2021).

The methanolic extract of santol fruit also exhibits bioactivity. At a concentration of 2000 µg per disc, it inhibits the growth of *Bacillus cereus* and *Staphylococcus aureus*, but it shows no activity against *Escherichia coli* (Poeaim and Pedklang, 2024). The peel extract has more potent antioxidant activity than the flesh, with IC₅₀ values of 30 µg/mL and 48 µg/mL for DPPH and ABTS assays, respectively. Furthermore, the peel extract achieves 76.37% tyrosinase inhibition at 1 µg/mL. Flesh and peel extracts display cytotoxic effects on HT-29 and Vero cell lines at 2000 µg/mL.

The fruit fly (*Drosophila melanogaster*) is a well-established model organism in research due to its genetic similarity to humans, making it an effective tool for studying human diseases. This model has been instrumental in advancing knowledge on genetic and molecular pathways in neurodegeneration, cancer, and metabolic disorders. (Panchal and Tiwari, 2017; Munnik *et al.*, 2022; Mirzoyan *et al.*, 2019). Fruit flies are particularly suited for large-scale studies due to their short life cycle, ease of maintenance, rapid reproduction, and low cost. For example, Chaweerak and Dechakhamphu (2020) found that santol peel extract was non-toxic to fruit flies at concentrations of 10 mg to 20 mg, and flies fed with the extract exhibited significantly lower triglyceride levels than the control group. The study investigated the nutritional value of santol fruit and evaluated the effects of crude santol flesh extract on *D. melanogaster*.

Materials and methods

Fruit fly stains

Wild-type flies (*D. melanogaster*) are characterized by distinct morphological traits, such as red eyes, a tan-colored body, and long, straight wings, which were obtained from Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand) were used in all experiments. The fruit flies were maintained in a humidity-controlled environment (60%) at 25 °C, with a 12-hour light-dark cycle. They were housed in test tubes containing a standard medium diet for fruit flies.

Plant collection and extraction

Santol fruits (*S. koetjape*) from the Pui-Fai variety were bought at a market in Bangkok, Thailand, in July 2024 (Figure 1). The fruits were washed with water, and the flesh was separated, cut into small pieces, and then dried at 45 °C for three days. Extraction was performed using methanol at a ratio of 1:5. The filtrate obtained by passing through Whatman No.1 filter paper was evaporated using a vacuum rotary evaporator. The resulting crude extract was stored in a glass bottle under vacuum until completely dried.

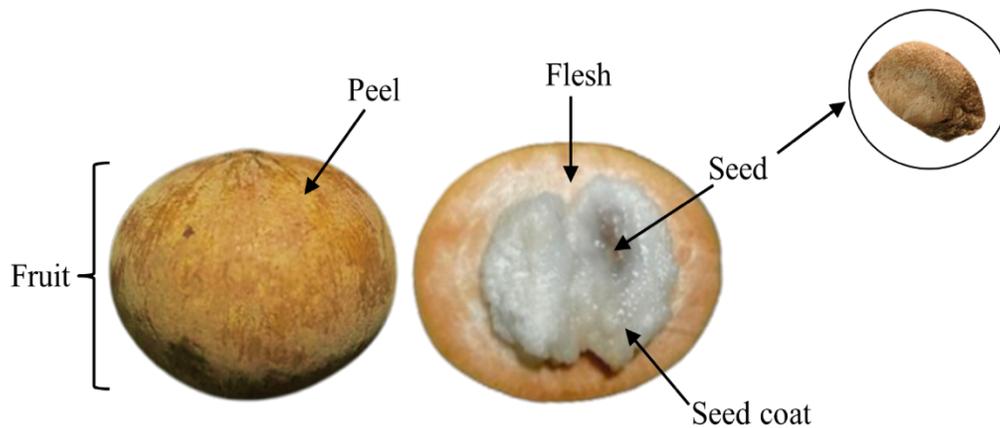


Figure 1. The structure of santol (*Sandoricum koetjape*)

Composition and nutrient content analysis

Four fresh santol fruit varieties were analyzed: E-lah (KT12) and Thongbaiyai (KT13) from Lopburi Province, and Puifai (KT34) and Puifai

(KT47) from Nonthaburi and Prachinburi Province, respectively. Composition and nutrient content, including fiber, macronutrients, vitamins, and minerals, were analyzed at Central Laboratory (Thailand) Co., Ltd. in Bangkok to determine the fruits' chemical properties.

Physical and biological responses

The experiment consisted of two groups: a treatment group, in which santol flesh extract was incorporated into the diets at concentrations of 20, 40, 60, 80, and 100 mg/mL, and the control group, which received a standard fruit fly medium diet without any added extract.

Body weight assay

Ten flies were used for each concentration, with five replicates per group. In the parental (P) generation, flies were incubated in the medium from adulthood until egg-laying (approximately 5 days). For the first filial (F1) generation, incubation starts at the egg stage and continues until adulthood, followed by an additional 3 days. After incubation, flies were anesthetized with ether, and their weight was measured using a digital analytical balance. (Mettler Toledo: MS105).

Egg laying rate

For each concentration, including the control, 10 flies (5 males and 5 females) were placed in tubes containing diets supplemented with crude extract, except for the control groups, which received only the standard diet. The flies were allowed to mate and lay eggs for 2 days, after which they were removed. The number of eggs on the diet surface was counted under a microscope.

Adult emergence rate

After egg-laying, the diets containing crude extract pupae were monitored until adult emergence. The total number of newly emerged adult flies was then counted and recorded.

Survival rate

Survival rates of flies were monitored throughout the study for all concentrations (20, 40, 60, 80, and 100 mg/mL), as well as for the control group. Dead flies were counted and recorded.

Climbing ability

The climbing ability of flies exposed to different concentrations of crude extract was assessed by placing them in empty tubes. The tubes were tapped to bring all flies to the bottom, and the time required to climb a 10 cm distance was recorded. The procedure adapted from Baenas and Wangner (2022) was repeated three times for each group, and the results were documented.

Statistical analysis

Statistically significant differences among groups were analyzed using One-Way ANOVA in Statistical Package for the Social Science (SPSS) version 17.0. with a 95% confidence level ($p \leq 0.05$). Data were presented as mean \pm standard deviation, and mean comparisons were performed using Tukey's test.

Results

Composition and nutrient content analysis

The nutrient content of santol fruits from the E-lah and Thongbaiyai varieties in Lopburi Province and the Puifai varieties in Nonthaburi and Prachinburi Provinces was compared with data for the yellow variety from the Republic of Honduras and an unspecified variety from India. Table 1 summarizes the nutrient content per 100 g. Santol fruits are low in calories, providing between 67.34 and 77.96 kcal, with 2.97 to 3.96 kcal derived from fat. The fruit is deficient in thiamine (vitamin B1), riboflavin (vitamin B2), and ascorbic acid (vitamin C). However, trace amounts of thiamine (<0.03 mg) are present in the Thongbaiyai variety and riboflavin (<0.025 mg) is found in the E-lah variety. Potassium is abundant, with concentrations ranging from 156.05 to 188.48 mg. The fruit also contains sodium (1.90–7.62 mg), calcium (4.25–10.04 mg), and iron (0.20–0.39 mg).

Body weight assay

Adult fruit flies in the parental (P) generation were weighed from adulthood to egg-laying (approximately 5 days). Across all groups, female flies consistently weighed more than males (Table 2). In the P generation, the average weights of flies on the control diet and diets supplemented with extract concentrations of 20, 40, 60, 80, and 100 mg/mL were 1.16 ± 0.20 , 1.09 ± 0.23 , 1.10 ± 0.24 , 1.11 ± 0.25 , 1.04 ± 0.19 , and 0.96 ± 0.22 mg per fly, respectively. For the

first filial (F1) generation, the corresponding weights were 1.15±0.20, 1.04±0.19, 1.09±0.23, 1.11±0.24, 1.04±0.19, and 0.94±0.21 mg per fly. These weight differences were statistically significant when compared to the control group.

Table 1. Comparison of nutritional content per 100 g of santol fruit across various varieties

Nutrients	Unit	Nutrient content per 100 grams of food					
		Yellow santol*	Unspecified type**	E-lah (KT12)	Thongbaiyai (KT13)	Puifai (KT34)	Puifai (KT47)
Ash	g	0.31	0.39	0.62	0.54	0.71	0.58
Calories	Kcal	N/A	N/A	72.45	77.96	76.78	67.34
Calories from fat	g	N/A	N/A	2.97	3.96	3.42	3.06
Carbohydrate	Kcal	N/A	N/A	16.49	17.74	17.58	15.31
Cholesterol	mg	N/A	N/A	N/D	N/D	N/D	N/D
Fat	g	0.10	0.52	0.33	0.44	0.38	0.34
Moisture	g	87.0	85.4	81.68	80.52	80.57	83.01
Protein	g	0.118	0.06	0.94	0.76	0.76	0.76
Saturated fat	g	N/A	N/A	0.08	0.16	0.1	0.08
Sugar	g	N/A	N/A	10.82	11.45	12.45	10.37
Fiber	g	0.10	1.26	4.51	3.53	3.6	2.57
Thiamine (Vitamin B1)	mg	0.045	N/A	N/D	<0.03	N/D	N/D
Riboflavin (Vitamin B2)	mg	N/A	N/A	<0.025	N/D	N/D	N/D
Ascorbic Acid (Vitamin C)	mg	86.0	N/A	N/D	N/D	N/D	N/D
Calcium	mg	4.30	5.38	10.04	7.09	4.25	4.74
Iron	mg	0.42	0.86	0.21	0.20	0.35	0.39
Potassium	mg	N/A	N/A	188.48	156.05	175.93	181.77
Sodium	mg	N/A	N/A	2.81	1.90	7.62	2.08
Phosphorus	mg	17.40	12.57	N/A	N/A	N/A	N/A
Carotene	mg	0.003	N/A	N/A	N/A	N/A	N/A
Niacin	mg	0.741	N/A	N/A	N/A	N/A	N/A
Pectin	mg	14.89	N/A	N/A	N/A	N/A	N/A

N/A: Not Applicable; N/D: Not Detected; Yellow santol from Honduras*; Unspecified type from India**

Table 2. The weight (mg/fly) of *D. melanogaster* exposed to diets with different concentrations of santol flesh extract in two generations

Weight of <i>D. melanogaster</i> (mg/fly)						
Concentrations (mg/mL)	Parental generation (P)			First filial generation (F1)		
	Female	Male	Average	Female	Male	Average
Control	1.37 ^a ±0.03	0.96 ^a ±0.01	1.16 ^a ±0.20	1.36 ^a ±0.02	0.95 ^a ±0.02	1.15 ^a ±0.20
20	1.32 ^d ±0.02	0.86 ^d ±0.01	1.09 ^e ±0.23	1.24 ^c ±0.01	0.85 ^d ±0.01	1.04 ^b ±0.19
40	1.35 ^c ±0.03	0.86 ^c ±0.01	1.10 ^e ±0.24	1.32 ^b ±0.04	0.85 ^c ±0.00	1.09 ^f ±0.23
60	1.36 ^b ±0.02	0.87 ^b ±0.01	1.11 ^d ±0.25	1.35 ^b ±0.01	0.86 ^b ±0.01	1.11 ^c ±0.24
80	1.24 ^e ±0.04	0.84 ^e ±0.01	1.04 ^b ±0.19	1.22 ^d ±0.02	0.85 ^d ±0.01	1.04 ^b ±0.19
100	1.18 ^f ±0.03	0.74 ^f ±0.03	0.96 ^a ±0.22	1.15 ^e ±0.01	0.73 ^c ±0.00	0.94 ^d ±0.21

The data are expressed as mean±SD. ($p<0.05$) indicates a statistically significant difference at a 95% confidence level, as determined by Tukey's test.

Egg laying rate

The egg-laying rate was evaluated after 2 days of mating and oviposition. The flies were then removed, and the number of eggs laid on the diet surface was counted under a microscope. Diets containing 60 mg/mL extract produced the highest egg-laying rate, whereas the 100 mg/mL concentration yielded the lowest rate (Figure 2). The 60 mg/mL diet also demonstrated a higher egg-laying rate than the control group.

Adult emergence rate

The adult emergence rate was recorded for flies reared on control and extract-supplemented diets. The control diet produced the highest adult emergence rate (Figure 3). Female emergence rates were consistently higher than male rates across all diets. Among the supplemented diets, 40 and 60 mg/mL concentrations exhibited higher emergence rates than other treatment groups but remained lower than the control.

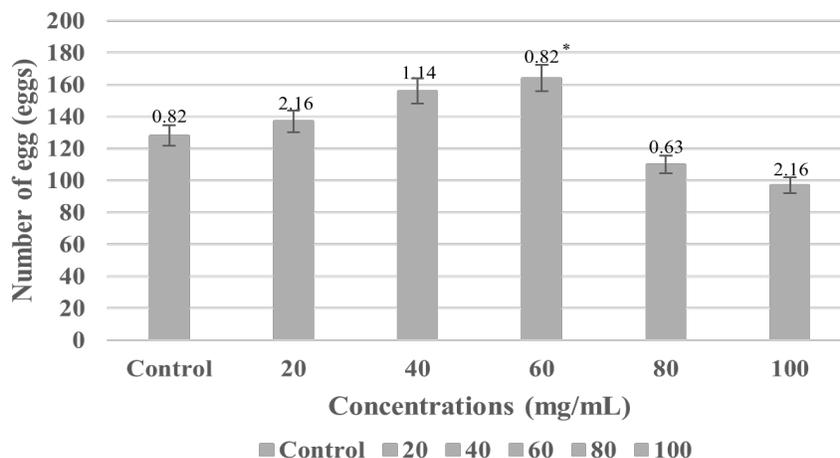


Figure 2. The egg-laying rate of *D. melanogaster* on different diets containing extract, comparing the control group: Bars represent the mean±SD. (*) ($p < 0.05$) indicates a statistically significant difference at a 95% confidence level determined by Tukey's test

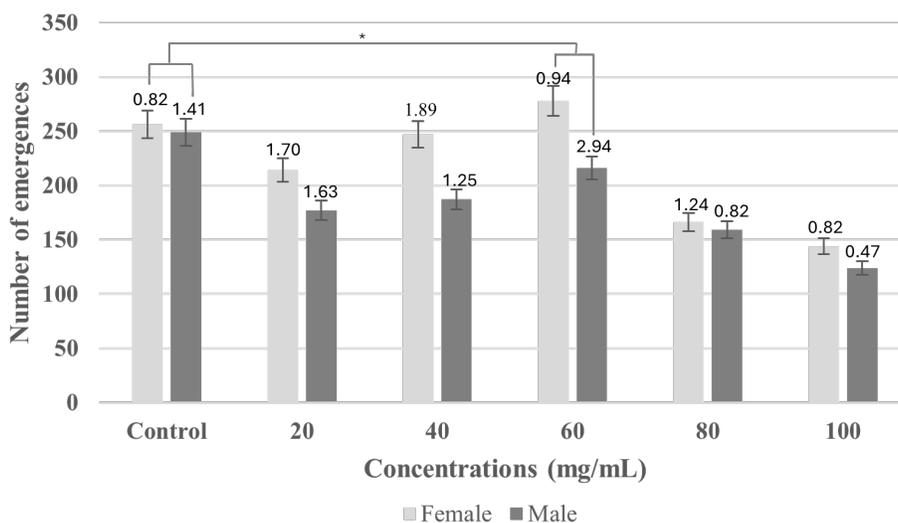


Figure 3. Adult emergence rate of *D. melanogaster* exposed to diets with different concentrations of santol flesh extract: Bars represent the mean±SD. ($p < 0.05$) indicates a statistically significant difference (*) ($p > 0.05$) when compared to the control group of the same sex, indicating no statistically significant difference at a 95% confidence level as determined by Tukey's test

Survival rate

Survival rates varied between the P generation and F1 generations. In the P generations, females exhibited lower survival rates than males. Diets supplemented with 80 and 100 mg/mL extract yielded survival rates below 50%. In contrast, in the F1 generation (recorded 3 days post-emergence from pupae), survival rates for both sexes exceeded 80% at all extract concentrations (Table 3).

Table 3. Survival rate (%) of *D. melanogaster* exposed to diets with different concentrations of santol flesh extract across two generations

Survival rate of <i>D. melanogaster</i> (%)						
Concentrations (mg/mL)	Parental generation (P)			First filial generation (F1)		
	Female	Male	Average	Female	Male	Average
Control	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00
20	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	93.33 ^a ±0.47	100.00 ^a ±0.00	96.67 ^a ±0.47
40	86.67 ^a ±0.47	93.33 ^a ±0.58	90.00 ^a ±0.81	93.33 ^a ±0.47	100.00 ^a ±0.00	96.67 ^a ±0.47
60	86.67 ^a ±0.47	93.33 ^a ±0.58	90.00 ^a ±0.81	93.33 ^a ±0.47	100.00 ^a ±0.00	96.67 ^a ±0.47
80	40.00 ^b ±0.82	53.33 ^b ±0.57	46.67 ^b ±1.24	100.00 ^a ±0.00	86.67 ^a ±0.58	93.34 ^a ±0.46
100	0.00 ^c ±0.00	13.33 ^c ±1.15	6.67 ^c ±0.94	100.00 ^a ±0.00	86.67 ^a ±0.58	93.34 ^a ±0.46

The data are expressed as mean±SD, and $p < 0.05$ indicates a statistically significant difference at a 95% confidence level determined by Turkey's test.

Climbing ability

The climbing ability of flies was assessed over a 10 cm distance. Males climbed faster than females, and climbing speed decreased as extract concentration increased (Figure 4). Flies reared on diets containing 100 mg/mL extract displayed the slowest climbing speeds.

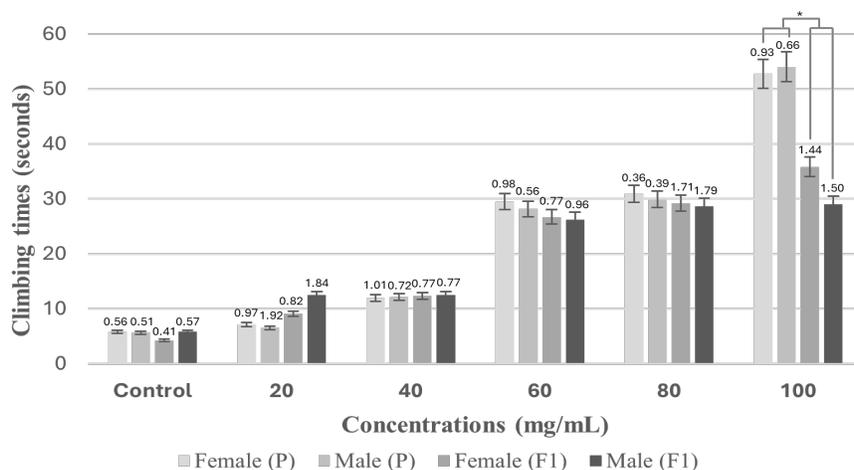


Figure 4. The climbing times of *D. melanogaster* exposed to diets with different concentrations of santol flesh extract across two generations: Bars represent the mean±SD. (*) ($p>0.05$) when compared to the control group of the same sex, indicating no statistically significant difference at a 95% confidence level and determined by Tukey's test

Life cycles

The generation time of fruit flies was affected by extract concentration. The control group had a generation time of approximately 10-11 days. Flies reared on diets containing 20, 40, and 60 mg/mL extract exhibited generation times similar to those of the control group. However, diets with 80 mg/mL extract extended the generation time to 11-13 days, and those with 100 mg/mL extract further delayed it by 13-15 days. These results are summarized in Table 4, with data for 20, 40, and 60 mg/mL concentrations shown in Figure 5.

Table 4. Generation times of *D. melanogaster* for the control group and on diets with santol extract at various concentrations

Concentrations (mg/mL)	Generation times (days)								
	Mate	Egg	1 st larva	2 nd larva	3 rd larva	Prepupae	Pupae	Adult	Sum
Control	2	1	1	1	1	2	1-2	2	10-11
20	2	1	1	1	1	2	1-2	2	
40	2	1	1	1	1	2	1-2	2	10-11
60	2	1	1	1	1	2	1-2	2	
80	2-3	2-3	1-2	1	1	2	1-2	2	11-13
100	2-3	2-3	1-2	1-2	1-2	2	1-2	2	13-15

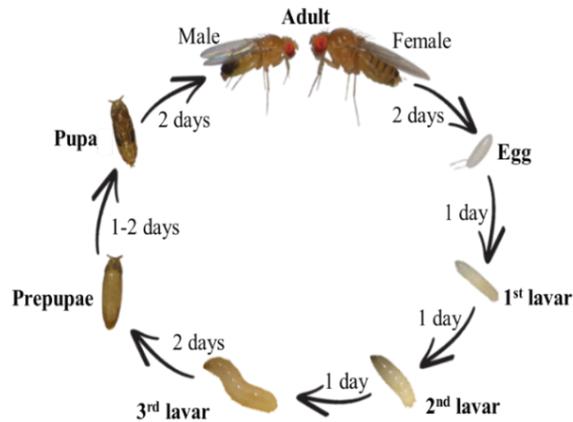


Figure 5. Generation times of *D. melanogaster* at santol flesh extract concentrations of 20, 40, and 60 mg/mL

Discussion

This study assessed the composition and nutrient content of santol (*S. koetjape*) fruit and investigated the effects of its extract on *D. melanogaster*. Key metrics included generation time, body weight, egg-laying rate, adult emergence rate, survival rate, and climbing ability. The experiment compared a control group, which received a standard diet, to treatment groups in which santol extract was added to diets at concentrations of 20, 40, 60, 80, and 100 mg/mL.

Nutritional analysis per 100 g revealed that santol is low in calories and lacks vitamins B1, B2, and C, unlike other sour fruits such as oranges and tamarinds, which are typically rich in organic acids like ascorbic acid, citric acid, malic acid, and tartaric acid (Shi *et al.*, 2022). However, santol contains unique triterpenes, such as sentulic acid and koetjapic acid (KA). KA, which is also found in a few other plants, exhibits notable anti-inflammatory and anticancer properties (Bailly, 2022). Additionally, santol is a rich source of potassium (156.05-188.48 mg), an essential mineral involved in fluid balance, nerve signaling, muscle contraction (Weaver, 2013), and the regulation of blood pressure through counteraction of sodium, promoting cardiovascular and bone health (Kim *et al.*, 2024; He and MacGregor, 2008).

The study found that adding santol extract to fruit fly diets caused several significant biological effects. Although the highest body weight occurred at a 60 mg/mL extract concentration, it remained lower than the control. Previous research has indicated that flavonoids, such as those in santol, can interfere with reproduction by disrupting hormone synthesis, including juvenile hormone and

ecdysone, which are critical for egg production (Puri *et al.*, 2022). The highest adult emergence rate was observed at 60 mg/mL, followed by 40 and 20 mg/mL, but all rates were lower than the control. This trend is consistent with studies on neem, a plant related to santol, where compounds like azadirachtin significantly reduced egg-laying and adult emergence in flies at concentrations of 1-3% (Kaur and Kocher, 2023).

Higher concentrations of santol extract (80 and 100 mg/mL) significantly reduced survival rates in the parental (P) generation, likely due to antimicrobial compounds in santol that disrupt the immune system or physiological functions of flies. Previous studies on plant extracts with bioactive properties (e.g., Isman, 2006) suggest that higher concentrations enhance toxicity, reducing survival rates.

Climbing ability was impaired in flies fed higher extract concentrations, with females being more affected than males. This could be attributed to sex-specific differences in octopaminergic neuron activity, which governs endurance and motor coordination in flies (Sujkowski and Wessells, 2018). Other insect studies have observed similar sex-based behavioral differences in response to bioactive compounds (Sujkowski and Wessells, 2018).

The generation time of flies was also affected by the extract. Flies fed diets with 80 mg/mL and 100 mg/mL concentrations showed extended generation times of 11-13 and 13-15 days, respectively, compared to the control group's 10-11 days. This delay is consistent with the effects of triterpenoids, compounds commonly found in the Meliaceae family with strong insecticidal properties, which act via mechanisms such as gastrointestinal poisoning, feeding deterrence, growth inhibition activities, and feeding deterrence (Gonzalez-Coloma *et al.*, 2011). These compounds likely disrupted developmental processes, resulting in slower growth, smaller size and lower body weight.

This study demonstrates that santol (*S. koetjape*) is a valuable source of potassium with potential human health benefits. However, adding santol extract to *D. melanogaster* diets negatively impacted key parameters, including climbing ability, adult emergence rates, and body weight in both the P and F1 generations. These findings suggest that the bioactive compounds in santol may interfere with growth, development, and reproduction, highlighting its potential application as a natural insect growth regulator in pest management. Future research should optimize santol extract for pest control applications while minimizing its effects on non-target species and exploring its broader uses in nutrition and pharmacology.

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